Available online freely at www.isisn.org

Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network

RESEARCH ARTICLE BIOSCIENCE RESEARCH, 2019 16(2): 1800-1807 **OPEN ACCESS**

CHROMagar™ O157 and gene sequencing for identification *Escherichia coli* from different sources

Israa AbdulJabbar Ibrahim[1], Sundas Abdul Muhamin Al-Hudari[2] and Mohammed Abbas Fayidh[2]

[1]Department of Microbiology, College of Science, Al-Karkh University of Science, Baghdad, **Iraq**.
[2]Department of Biology, Ibn Al-Haitham College of Education for Pure Science, Baghdad University, **Baghdad, Iraq.**

***Correspondence: israa_ibrahim66@yahoo.com** **Accepted: 10 Jan**. 2019 **Published online:** 02 June 2019

E. coli O157 was responsible for death due to consumption of contaminated foods. This study was to investigate prevalence of *E. coli* O157 in some leafy vegetables, minced beef meat and children diarrhea stool, by means of rapid method of isolation CHROMagar™ O157 and gene sequencing by using universal primer for 16S rRNA gene (27F and 1492R). The results of this analysis showed that proximity and the genetic dimension among the Iraqi isolates collected from different sources among themselves and as compared to the NCBI isolated samples, with 99% compatibility values with isolates recorded in NCBI. The phylogenic tree of the current study reflects a high diversity between those samples which are separated into 10 nodes, and 4 main groups (A1, A2, B1, B2).

Keywords: Contaminated foods, *Escherichia coli*, phylogenic tree

INTRODUCTION

Escherichia coli is a common inhabitant of the intestinal tract of man and warm-blooded animals and it is also found in the environment (water, soil). However, within the species, there are 7 strains or categories that cause intestinal and extra-intestinal human infections (EPEC, EHEC or STEC, ETEC, EIEC, EAEC, DAEC, UPEC) (Brenner, 2005; Hussain, 2015). *Escherichia coli* O157:H7 serotype is one of the Shigatoxin–producing types of *E.coli* (Lim, *et al*, 2010). Utilizing 16S rRNA sequences, various bacterial genera and species have been reclassified and renamed (Woo *et al*, 2008). Most diseases caused by these bacteria are a result of the utilization of less cooked meat and unpasteurized dairy products and drinking water contaminated with feces (Rey *et al*, 2006). A study by Delaquis *et al* (2007) reported Leafy vegetables (lettuce and spinach) have been implicated in several outbreaks of foodborne disease caused by *Escherichia coli* O157:H7. Yalcin *et al*. (2017) surveyed sampling of food (restaurants, shop, market) in Istanbul City, show that the prevalence of *E coli O157* in food was 2%, but its prevalence increased in April and May. Other report from some markets located in Riyadh City, uncovered the incidences of *E. coli* O157:H7 in ground beef, beef burgers, beef sausage, ground chicken and chicken burgers as follows: 5%, 10%, 0.0%, 5% and 0.0%, respectively (Bosilevac, *et al* 2015). Due to the modest local study trying to determine the prevalence of *Escherichia coli O157* in different sources, we aimed to determine the following: 1stthe percentage of this pathogen in beef meat, leaf vegetables and children diarrhea stool, collected from a certain local place by using CHROMagar™ *O157* and 2nd sequencing with universal primer (27F and 1492R) to determine the relation among isolates.

MATERIALS AND METHODS

Specimens

In this study, 60 *E. coli* isolates were analyzed;- 20 *E. coli* isolates were obtained from minced beef meat of the local market, 20 isolates from leafy vegetables (veg.) and the other twenty bacterial isolates were collected from children diarrhea from Children Hospital Center in Baghdad city**.** Collecting samples was carried out from October 2015 to February 2016.

Isolation and identification

All samples in the first collection were cultured on MacConkey agar and Eosin methylene blue agar plates. Subcultures in CHROMagar™ O157 [Easier detection: *E. coli* O157 are detected as mauve colonies among blue *E. coli spp*. (The Chromogenic Media Pioneer- France)].

β-Lactamases detection

CHROMagar™ESBL Chromogenic medium for overnight detection of gram-negative bacteria producing extended spectrum beta-lactamase [ESBL (Extended Spectrum β-Lactamases) are enzymes that mediate resistance to penicillin, extended-spectrum third generation cephalosporin (C3G) and monobactam].

Extraction of total DNA

Selected sample- Eight samples were selected on the CHROMagar™ O157 and CHROMagar™ ESBL (Table 1).

Table 1 Selected sample

No.	Source	result
1	Meat	Sensitive
2	Veg	Sens.
3	Stool	Sens.
4	Meat	Resistance
5	Veg.	Resis.
6	Stool	Resis.
7	Meat	O157 +ve
8	Veg.	O157 +ve

DNA extraction- Total DNA of bacterial isolates was extracted by using Promega Genomic DNA Purification Kit (A1120).

PCR Amplification

The 16S rRNA gene was amplified by using the universal primer 27F (5'-AGA GTT TGA TCM TGG CTC AG-3') and 1492R (5'-CGG TTA CCT TGT TAC GAC TT-3') (Jiang *et al*., 2006). The PCR amplification is performed in a total volume of 25µl containing 1.5µl DNA, 12.5µl Green Master Mix PCR (Promega, USA), 1µl of each primer 10 pmol then nuclease-free water is added into a tube to a total volume of 25µl. Thermo cycling conditions were as follows: initial denaturation at 4 min at 95°C, followed by 30 cycles of denaturation 95 °C for 30 sec, annealing at 55°C for 40 sec, extension at 72 °C for 90 sec and a final extension of 72°C for 10min. The PCR products were separated on 1% agarose gel. The gel is left to run for 90min with a 100volt/50 mAmp current. Following electrophoresis, visualization was conducted with a UV transilluminator after ethidium bromide staining.

Sequencing

The sequencing of 16S rRNA gene was performed at Macrogen Inc., by using their ABI 3730xl genetic analyzer (Applied Biosystems, US). Homology search was conducted by using Basic Local Alignment Search Tool (BLAST) program which is available at the National Center Biotechnology Information (NCBI) website: (http://www.ncbi.nlm.nih.gov) and BioEdit program. The results were compared with data obtained from Gene Bank published ExPASY program which is available at the NCBI website.

RESULTS AND DISCUSSION

Identification by using CHROMagarTM O157 and CHROMagarTM ESBL

The identification by using CHROMagar™ *O157* revealed that 2 out of 60 samples were positive for *E. coli O157*, and CHROMagar™ ESBL showed 70% of them to be beta-lactamase production (Table 2). Chromogenic media have been used for identification of microorganisms in clinical and food sample (Perry and Freydiere 2007; Ganda *et al*., 2016).

A number of local studies depended on different methods to identify *E. coli O157* such as: Sorbitol MacConky media and LatexO157 Serotyping Kits (Al-Rudha *et al*., 2016; Al-Rubaey, 2016; Al-Shedidi, 2017), Chromagar *E. coli* O157:H7 (Al-Oebady, 2017).

A previous local study showed out of the total 136 *E. coli* isolates, 58.82% were found to be ESBL producers (Hamdoon, 2011), 27 isolates from Infantile Diarrhea in Kut City (96.4%) were able to produce extended-spectrum β-lactamases (ESBLs) (Shamki, *et al*., 2012).

Table 2 Test sample CHROMagar^{TM} O157 and CHROMagar^{TM} ESBL results

Sample	O157 (O.)	ESBL	
		Resistance (R.)	Sensitive (S.)
children diarrhea stool20	-	17	3
minced beef meat20	1	18	2
leafy vegetables20	1	7	13
Total 60	2	42 (70%)	18 (30%)

Identification by using universal primer

Figure 1 shows the positive results for identification *E. coli* strain by using 16S rRNA gene [universal primer 27F (5'-AGA GTT TGA TCM TGG CTC AG-3') and 1492R (5'-CGG TTA CCT TGT TAC GAC TT-3')]. Many previous researches were dependent on universal primer for diagnosis certain species from different sources (Jiang, *et al*, 2006; Frank *et al*, 2008; Piterina *et al*, 2010; Senthilraj *et al*, 2016).

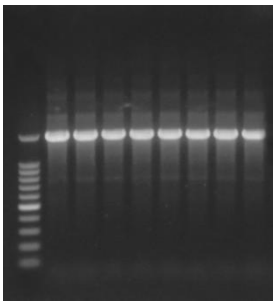

Figure. 1. Agarose gel electrophoresis of 16S rRNA gene (1504bp), along a 100-bp DNA ladder (Promega, US).

The identification *E. coli* isolates by using 16S rRNA gene (universal primer 27F and 1492), showed *E. coli* in different groups, as follows by:-

A First group isolated from Meat S. and Meat R.

Meat was collected from local market. Meat is among the public health concerns associated with microbial pathogens. Contaminated meat can cause food-borne illnesses that can lead to serious medical conditions (WHO, 2008). The results obtained showed that 5 variations: three Transversion and two Transition -when comparing between isolate 1 and 4 - have shown 99% compatibility (Table 3).

Partial sequences (1504bp bp) of the 16S rRNA gene of both isolates 1 and 4 - have been registered in GenBank with accession numbers ID: KX354348.1 (bacteria isolates of food stuff from India, 2016) and ID: MF370888.1 (bacteria isolates of fly from China, 2017), respectively (Patial, P., Jandaik, S., 2016; Zhang, J. 2017).

Second group isolated from fresh vegetables S. and vegetables R.

Fresh vegetables were randomly collected from different markets of Baghdad city for possible isolation of the pathogens. The results obtained showed that 8 variations five Transversion and three Transition - when comparing between isolate 2 and 5 - have shown 99% compatibility (Table 4),

Both isolates 1 and 4 have been registered in GenBank with accession numbers ID: KY780359.1 (antibiotic-resistant isolates of hospitals from Taif, Saudi Arabia, 2017) and ID: MF957269.1 (bacteria isolates of feces animal host "*Bos taurus*" from USA,2017), respectively (Hassan and Farag, 2017; Teng, 2007).

Third group isolated from Stool S. and Stool R.

The results obtained showed that 5 variations: three Transversion and two Transition - when comparing between isolate 3 and 6 - have shown 99% compatibility (Table 5), under sequence ID: KP244257.1 (carbapenem-resistant Gram negative bacteria of urine from Tamil Nadu-India, 2014) and ID: KY780359.1 (antibiotic-resistant isolates of hospitals from Taif, Saudi Arabia, 2017), respectively (Ramesh, *et al*., 2014;Hassan and Farag, 2017).

Fourth group isolated from meat and vegetables (*O157* +ve).

The results obtained showed that 19 variations: seven Transversion and twelve Transition - when comparing between isolate 7 and 8 - have shown 99% compatibility (Table 6), under sequence ID: HQ658163.1 (organism="*Escherichia coli* O157 :H7"Vietnam, 2010) (Hoang and Le, 2010).

Transition and Transversion are the changes that have a role in distinguishing Iraqi isolates from other ones, indicating the genetic dimension and proximity, as well as changing the phenotypic properties, virulence factors, antimicrobial resistance, and change of bacteria's ability to

survive.

Table 3 Represent the type of polymorphism of 16S rRNA gene from *Escherichia coli*, for isolates1 and 4.

No. of sample	Type of substitution	Location	Nucleotide	Sequence ID
Isolate1	Transversion	92	G>T	**ID: KX354348.1**
	Transversion	1025	A>T	
	Transversion	1051	G>T	
Isolate4	**Transition**	**1101**	**A>G**	**ID: MF370888.1**

Table 4 Represent the type of polymorphism of 16S rRNA gene from *Escherichia coli*, for isolates 2 and 5.

No. of sample	Type of substitution	Location	Nucleotide	Sequence ID
Isolate2	Transversion	217	A>T	**ID: KY780359.1**
	Transversion	220	A>T	
	Transition	969	G>A	
	Transition	982	G>A	
	Transversion	1035	A>C	
	Transversion	1055	G>T	
Isolate5	Transition	919	C>T	**ID: MF957269.1**
	Transversion	**1072**	**A>C**	

Table 5 Represent the type of polymorphism of 16S rRNA gene from *Escherichia coli*, for isolates 3 and 6.

No. of sample	Type of substitution	Location	Nucleotide	Sequence ID
Isolate 3	Transition	226	C>T	ID: KP244257.1
	Transversion	1051	G>T	
Isolate 6	Transition	982	G>A	ID: KY780359.1
	Transversion	984	T>A	
	Transversion	985	G>T	

Table 6 Represent the type of polymorphism of 16S rRNA gene from *Escherichia coli*, for isolates 7 and 8

No. of sample	Type of substitution	Location	Nucleotide	Sequence ID
Isolate 7	Transition	136	A>G	**ID: HQ658163.1**
	Transversion	241	A>T	
	Transversion	244	A>T	
	Transversion	264	T>A	
	Transition	704	A>G	
	Transition	877	A>G	
	Transition	993	A>G	
	Transversion	997	C>G	
	Transition	1001	C>T	
	Transversion	1012	T>A	
	Transition	1029	T>C	
Isolate 8	Transition	136	A>G	**ID: HQ658163.1**
	Transition	704	A>G	
	Transition	877	A>G	
	Transition	993	A>G	
	Transversion	997	C>G	
	Transition	1001	C>T	
	Transversion	1012	T>A	
	Transition	1029	T>C	

Phylogenetic analysis

The phylogenetic tree is diagrammatic by Molecular Evolutionary Genetics Analysis (MEGA) software version 6.0 is shown in figure (2). The 8 isolated sequences have shown 99% compatibility, by comparing the isolates collected from different sources (Meat, vegetables, and Stool). The neighbor-joining tree was constructed for phylogenetic analysis. These alignments revealed the genetic distance and other global strains by partial sequence similarity in 16S ribosomal RNA gene for translating specific region. The genetic dimension between Iraq isolates and the ones of the world is detailed according to the phylogenetic tree and the comparison table. Sample 2, 4, 7 and 8 isolated from veg. (S.), meat (R.), meat (O.) and veg. (O.) respectively showed that the genetic dimension was by 0.1 as it is close to Indian isolated from strain="BT_HNGU_12" by ID: KX984112.1. Sample 6 isolated from stool (R.) shows that the genetic dimension was by 1.2 as it is close to Korean one isolated from strain="chicken11" ID: JX041515.1. Concerning sample 3 isolated from stool (S.), the genetic dimension was by 0.4 as it is close to Indian isolated from strain="Pk1" ID: KX354348.1. Regarding sample 5 isolated from veg. (R.) the genetic dimension was by 1.2 as it is close to Vietnam ID: HQ658163.1. Whereas with sample 1 isolated from meat (S.) the genetic dimension was by 0.8 as it is close to the Japanese isolated from strain="JCM 1246" ID: LC050175.1. Many researchers, as a matter of fact, use universal gene 27F and 1492R to distinguish *E. coli* strain (Frank *et al*., 2008; Isenbarger *et al*., 2008; Suardana 2014, Ibrahim, 2016). The results of this analysis showed that samples isolated from Meat, vegetables, and Stool respectively, are closely related among them. The sequences showed the highest identity, i.e. (99%).

The comparison between *Escherichia coli* isolated from Meat, vegetables, and Stool, recorded in the National Center Biotechnology Information (NCBI) and which were isolated from different source have under sequence (ID: AB035925.1, ID: AM184233.1, ID: AB035924.1, ID: AY513502.1, ID: HM007589.1**,** ID: HQ658163.1**,** ID: KX984112.1**,** ID: MG663511.1**,** ID: KY120324.1**,** ID: KX354348.1**,** ID: KP235234.1**,** ID: LC050175.1**,** ID: LN875375.1**,** ID: KX421365.1, ID: KJ410177.1**,** ID: HG941666.1**,** ID: JX041515.1**,** ID: KP789329.1**,** respectively with source of isolation and showed compatibility of 99% and score (1887-1793), and expect 0.0 with gene bank.

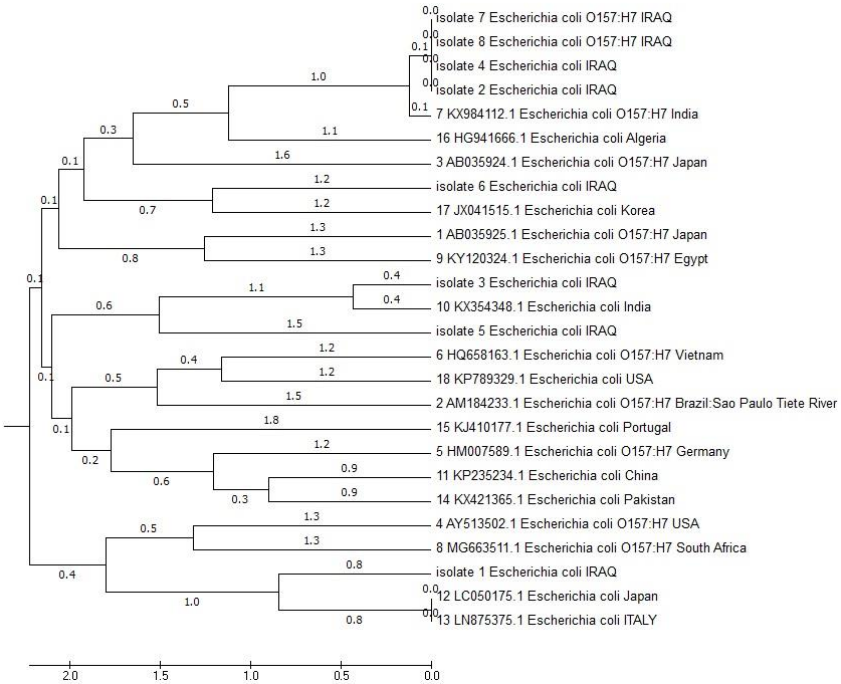

Figure 2. Neighbor-joining tree *Escherichia coli* of 16S rRNA gene.

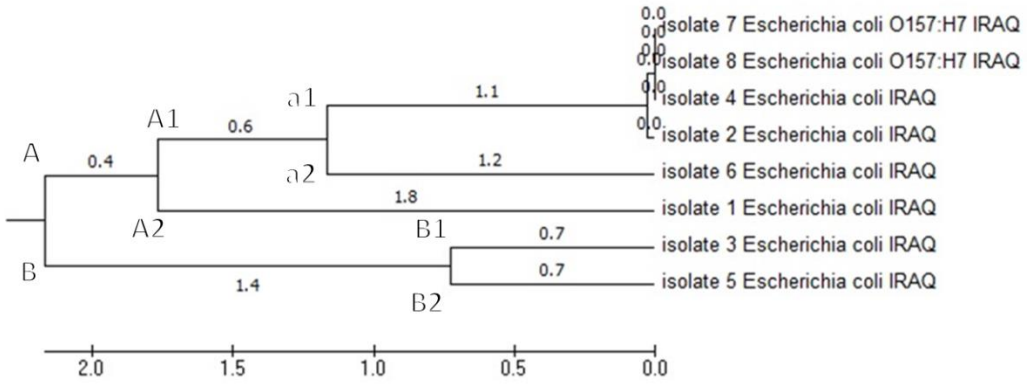

Figure 3. **Neighbor-joining tree *Escherichia coli* from Iraqi isolates of 16 S rRNA gene.**

Figure (3) revealed only sequences that showed the highest identity (>98%) and maximum coverage (>99%). Twenty four strains from GenBank database related to certain isolates from different source sample.

When the Iraqi isolates were compared among them. The tree was with 10 nodes and 8 Tips. The data is separated into 2 groups (A and B). The phylogenic tree of the current study reflects a high diversity between isolates. It is clear that cluster A1 shows isolates 8, 7, 4 and 2 isolated from veg. (O.), meat (O.), meat (R.), and veg. (S) respectively. The resulting tree, still mixes non-pathogenic *E. coli O157* with other *E. coli* isolates, and does not separate all pathogenic strains from commensal strains this phenomenon also reported by Lukjancenko *et al*.,(2010).

The genetic dimension was by 1.1 having a different pattern compared with isolate 6 isolated from stool (R.) where the genetic dimension was by 1.2 in the same group of A1 node. However, A2 group isolate 1 isolated from meat (S.) where the genetic dimension was by 1.8, suggested the same ancestors of A node. Furthermore, what appeared in cluster B, precisely isolate 3 isolated from stool (S.) and isolate 5 isolated from vegetables (R.) proved that they did not have differences in genetic dimension as it was by 0.7; this result suggested isolate 3 and isolate 5 to be from the same ancestors.

CONCLUSION

Culture in selective media and enzymatic interactions can be used to identify process of genus or species but is difficult to determine the strain. This study reports the use of universal 16S rRNA gene sequencing after first isolates by using CHROMagarTM media apart from its sufficiency for determine certain strain. Such primer is not sufficient to determine the differences between beta-lactamase production and not production though.

The *Escherichia coli* isolates 8, 7, 4, and 2 collected from vegetable and meat, respectively, were originated from the same source according to the analysis of 16S rRNA gene. Also, isolates 3 and 5 collected from stool and vegetable originated from the same source according to the analysis of 16S rRNA gene. The efficiency of genetic methods in the detection of accurate and rapid bacterial isolates proved the closeness of the Iraqi isolates *E. coli* O157 to the other isolates (India, Saudi Arabia, and Vietnam).

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

ACKNOWLEGEMENT

The authors do thank all the staff in the Department of Biology, Ibn Al-Haitham College of Education for Pure Science, Baghdad University and Wahj Aldna Co. for Qualification and Training, Baghdad, Iraq.

AUTHOR CONTRIBUTIONS

Israa AJ Ibrahim designed the experiments and also wrote the manuscript. Sundas AM Al-Hudari and Mohammed A Fayidh, performed the sample collection, isolation of bacteria, 16S rRNA gene sequences and data analysis. All authors read and approved the final version.

REFERENCES

Al-Oebady M.A.H., 2017. Identification of *E.coli* O157:H7 in Intestinal and Urinary Tract Infection in Samawah City. J. Babylon University/Pure Appl. Sci., 25(2):455-460.

Al-Rubaey M.G.A., 2016. Detection of *Escherichia coli* and *E. coli O157* in Veal Mincemeat in Baghdad. J. Veter. Med. Sci., 7 (1):127- 131.

Al-Rudha, A.M.H., Al-Rubaie, E.M., Khalil, N.K., 2016. Distribution of *E.coli* O157:H7 in fecal and urine samples of cattle. Iraqi J. Veter. Med., 40 (1): 79-82.

Al-Shedidi A.M.S. 2017. The antimicrobial role of emulsifying salts against *E. coli* O157:H7 contaminated local markets soft cheese of Baquba city. Iraqi J. Veter. Med., 41(1): 5-10.

Bosilevac, J.M.1., Gassem, M.A., Al Sheddy, I.A., Almaiman, S.A., Al-Mohizea, I. S., Alowaimer, A., Koohmaraie, M., 2015. Prevalence of *Escherichia coli* O157:H7 and Salmonella in camels, cattle, goats, and sheep harvested for meat in Riyadh. J. Food Prot. 78(1):89-96.

Brenner, D.J., Krieg, N.R., Staley, J.T., Garrity G.M., Boone, D.R., De Vos, P., Goodefellow, M., Rainey, F.A., Schleifer, K.H. 2005. Bergey's Manual of Systematic Bacteriology,. 2nd ed. Michigan State University East Lansing: Springer.

Delaquis, P., Bach, S., Dinu, L-D., 2007. Behavior of *Escherichia coli* O157:H7 in Leafy Vegetables. J. Food Prot. (70) 8: 1966–1974.

Frank, J.A., Reich, C.I., Sharma, S., Weishaum, J.S., Wilson, B.A., Olsen, G.J., 2008. Critical Evaluation of Two Primers Commonly Used for Amplification of Bacterial 16S rRNA Genes. Appl. Environ. Microbiol., 74 (8): 2461–2470.

Ganda, E.K., Bisinotto, R.S., Decter, D.H., Bicalho, R.C., 2016. Evaluation of an On-Farm Culture System (Accumast) for Fast Identification of Milk Pathogens Associated with Clinical Mastitis in Dairy Cows. PLOS ONE DOI: 10.1371/journal.pone.0155314 May 13.

Hamdoon, A.M., 2011. Detection of extended spectrum B-lactamase in *E. coli* from clinical samples. Ann. Coll. Med. Mosul., 37 (1 & 2): 122-128.

Hassan, M.M., Farag, M.M., 2017. Molecular epidemiology and genetic characterization of *Escherichia coli* antibiotic-resistant isolates from hospitals in Taif, Saudi Arabia. http://www.ncbi.nlm.nih.gov

Hoang,H.P., Le,H.Q., 2010. Cloning and sequencing of 16S rRNA of *Escherichia coli* O157:H7 Submitted (22-NOV-2010) Khoa Sinh-KTNN, DHSP TN, DHSP TN, 20 Luong Ngoc Quyen, Thai Nguyen, +84 +84280, Vietnam. http://www.ncbi.nlm.nih.gov

Hussain, T. 2015. An introduction to the Serotypes, Pathotypes and Phylotypes of *Escherichia coli*. Int. J. Microbiol. Allied Sci. (IJOMAS) 2 (1): 9-16.

Ibrahim, I.AJ., 2016. 16S rRNA Gene Sequencing for Identification of Some Enterobacteriaceae Species Isolated from Tigris River. Al-Mustansiriyah J. Sci. 27(3):13-16.

Isenbarger, T.A., Finney, M., Rios-Velazquez, C., Handelsman, J., Ruvkun, G. 2008. Miniprimer PCR, a new lens for viewing the microbial world. Appl Environ Microbiol 74, 840–849.

Jiang, H., Dong, H., Zhang, G.,Yu,B., Chapman, L.R., Fields, M.W. 2006. Microbial Diversity in Water and Sediment of Lake Chaka, an Athalassohaline Lake in Northwestern China. Appl. Environ. Microbiol. 72 (6): 3832–3845.

Lim J.Y., Yoon J.W., Hovde C.J., 2010. A Brief Overview of Escherichia coli O157:H7 and Its Plasmid O157. J. Microbiol. Biotechnol. 20 (1): 5–14.

Lukjancenko, O., Trudy, M., Wassenaar, T.M., Ussery, D.W., 2010. Comparison of 61 Sequenced *Escherichia coli* Genomes. Microbiol. Ecol., 60:708–720.

Patial, P., Jandaik, S., 2016. Molecular characterization of isolates isolated from food stuff Submitted (01-JUN-2016) Dept of Biotechnology, Shoolini University, Bhajol, Solan, Himachal Pradesh 173212, India. http://www.ncbi.nlm.nih.gov

Perry, J.D., Freydiere, A.M., 2007. The application of chromogenic media in clinical microbiology. J. Appl. Microbiol. 103(6):2046–55. PMID: 18045388.

Piterina, A.V., Bartlett, J., Pembroke J.T., 2010. Molecular Analysis of Bacterial Community DNA in Sludge Undergoing Autothermal Thermophilic Aerobic Digestion (ATAD): Pitfalls and Improved Methodology to Enhance Diversity Recovery. Diversity, 2, 505-526; doi:10.3390/d2040505.

Ramesh, N., Ramkumar, S., Prasanth, M.,

Gothandam, K.M., Karthikeyan, S. 2014. Characterization of carbapenem-resistant Gram negative bacteria from Tamil Nadu, Submitted (06-SEP-2014) School of Bio Sciences and Technology, VIT University, Vellore, Hitech Diagnostic Laboratory-Chennai, Vellore, Tamil Nadu 632014, India. http://www.ncbi.nlm.nih.gov

Rey, J., S´anchez, S., Blanco, J.E., de Mendoza, J.H., de Mendoza M. H., Garcia, A., Gil, C., Tejero, N., Rubio, R., Alonso, J.M., 2006. Prevalence, serotypes and virulence genes of Shiga toxin-producing *Escherichia coli* isolated from ovine and caprine milk and other dairy products in Spain. International Journal of Food Microbiol. (107) 2: 212–217.

Senthilraj, R., Prasad, G. S., Janakiraman, K., 2016. Sequence-based identification of microbial contaminants in non‑parenteral products. Brazil. J. Pharm. Sci. 52(2):329-336.

Shamki, J., Al-Charrakh, A.H., Al-Khafaji J.K., 2012. Detection of ESBLs in Enteropathogenic *E. coli* (EPEC) Isolates Associated with Infantile Diarrhea in Kut City. Med. J. Babylon, 9 (2): 403- 412.

Suardana, I. W., 2014. Analysis of Nucleotide Sequences of the 16S rRNA Gene of Novel *Escherichia coli* Strains Isolated from Feces of Human and Bali Cattle. J. Nucl. Acids, Article ID 475754, 7 pages.

Teng, L. (2007). Submitted (13-SEP-2017) Animal Sciences, University of Florida2055 MOWRY ROAD, Emerging Pathogens Institute, Room 200, Gainesville, FL 32608, USA. http://www.ncbi.nlm.nih.gov

Woo, P.C.Y., Lau, S.K.P., Teng, J.L.L., Tse, H., Yuen, K.Y. 2008. Then and now: use of 16S rDNA gene sequencing for bacterial identification and discovery of novel bacteria in clinical microbiology laboratories. Clin. Microbiol. Infect. 14 (10): 908–934.

World Health Organization, 2008. Foodborne Disease Outbreaks "Guidelines for Investigation and Control "ISBN 978 92 4 154722 2 (NLM classification: WC 260), Printed in France.

Yalcin, S., Alcay, A.U., Yuzbasioglu, G., Cakmak, B., Saglam, A. 2017. A study on prevalence of *E. coli O157* with a Verified method in foods. Turk. J. Agri. Food Sci. Technol. 5(4): 294-298.

Zhang, J. 2017. The mcr-1 in flies and bacteria isolates of fly from China Submitted (19-JUN-2017) College of Veterinary Medicine, Yangzhou University, East of Wenhui Road No. 12, Yangzhou, Jiangsu 225009, China. http://www.ncbi.nlm.nih.gov.